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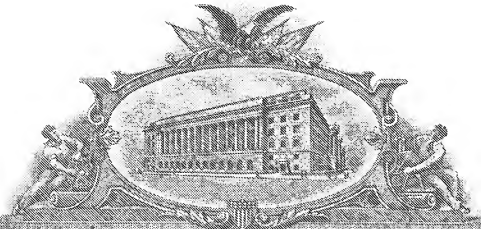

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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☐ *Additional inventors are being named on the____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

FRUCTANS: INCLUSIVE OF INULINS, LEVANS, AND RELATED FRUCTOFURANOSIDES AS SUBTERRANEAN TERMITE FEEDING ATTRACTANTS IN TERMITE BAITING SYSTEMS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages*	45	☐ CD(s), Number	
☐ Drawing(s) *Number of Sheets*		☐ Other (specify)	
☐ Application Data Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☒	Applicant claims small entity status. See 37 CFR 1.27.	FILING FEE AMOUNT ($)
☒	A check or money order is enclosed to cover the filing fees	
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE

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Date 3-22-04

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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FRUCTANS: INCLUSIVE OF INULINS, LEVANS, AND RELATED FRUCTOFURANOSIDES AS SUBTERRANEAN TERMITE FEEDING ATTRACTANTS IN TERMITE BAITING SYSTEMS

Subterranean termites are the single most important structural pest in the United States. Each year millions of dollars are spent on subterranean termite prevention, control, and damage repair. What makes these insects such significant pests is the fact that their diet consists solely of wood and other cellulose materials. In the natural environment subterranean termites provide the valuable service of recycling (consuming and digesting) dead and decayed wood and returning those nutrients to the soil. However, when humans replace natural food resources with wood homes and other structures the termites' habit of consuming wood becomes a significant problem.

There have been a number of methods used to prevent subterranean termite attack on wood structures. The oldest and most commonly used method has been the application of liquid termiticide to the soil around the structure. However, this method requires that large amounts of dilute pesticide be applied to and beneath the foundation. Many homeowners are not comfortable with the invasive applications methods of liquid treatment or the toxicity of the chemicals being placed in their immediate living environment. Therefore, a more novel approach to termite control was developed in the 1990s to eliminate the unpleasant attributes of liquid termiticide application. This approach involved the installation of a termite baiting systems.

Termite baiting systems are applied by installing plastic stations that contain wood blocks (monitors) into the ground around the structure. Termites tunneling in the soil encounter these stations and begin to consume the wood. Theoretically, the termites then recruit additional termites to the bait station so that large numbers of termite workers begin feeding on the monitors. A pest management professional checks the stations monthly. When he or she finds a station that has been "hit" by termites the wood monitor is replaced with a cellulose bait containing a toxicant. The termites in the station consume the bait and then feed it to their nest mates. In this way the toxicant is spread throughout the termite colony and the colony dies.

In spite of the environmental friendliness and unobtrusiveness of termite bait systems, they have several limitations. Not the least of which is the small presence an individual station has in the outdoor environment. Because there is no way to direct termite foraging into the bait system it may take considerable time for termites to discover the

stations. In addition, subterranean termites are known to prefer feeding on particular types of wood and wood in certain conditions of decay. In other words, wood whose *chemical composition* tastes better than other wood available in the same area. If these better tasting food sources are in the same location where baiting is being attempted, the preferred food sources will have a significant impact on the bait system efficacy.

Ideally, the bait system would contain the most attractive, best tasting food resource in the area. Then we could expect subterranean termites to recruit to the bait stations in large numbers and find the bait so delicious that they would eat it preferentially over other competing food resources. For this to be the case, an important question that needs to be addressed. Is there a chemical, or group of chemicals that can be added to a termite monitor and /or bait that would enhance its attractiveness to foraging termites?

As stated above, the primary food source for subterranean termites is wood. Subterranean termites play a significant role in the ecosystem because they recycle woody materials. However, woody materials are, because of their chemical composition, difficult to degrade. Wood is composed of cellulose, which consists of beta-linked polymers of glucose and lignins. These polymers form complex organic matrices that are relatively difficult for organisms to digest. Only two major groups of organisms possess the capability to degrade woody materials. These are free-living fungi and the microorganisms contained in the digestive track of termites.

Termite microbes require the anaerobic environment provided in their hindguts to digest the cellulose/lignin complex into smaller carbon units and nutrient molecules. The byproducts of wood digestion consist of smaller units of cellulose and lignins. The sub units of digested cellulose are composed of heteropolymers or heteropolysaccharides. These molecules are beta-1,4-linked cellulose units of variable molecular weight. The larger polysaccharides are not water soluble, but smaller units (approximately 5000 daltons) are water soluble.

It is possible that some of these water soluble polymers such as inulin (approximately 35 beta-1.4 linked hexose units; 5880 daltons) might serve as feeding attractants for termites, since they represent less complex molecules that might be digested more easily by the termite microbial system. We have discovered in choice test experiments that cellulose (paper) diets spiked with very low concentrations of inulin are significantly more attractive to termite workers compared to other diet sources tested. We propose to seek patent rights of all low molecular weight beta cellulose polymers consisting of up to 75 hexose units, with molecular weights ranging between 1000 to 12,600 daltons, which are water or slightly water soluble, as subterranean termite attractants for use in termite baiting systems.

If an INVENTION, provide a complete description and identify and describe the novel or unusual features. Use extra sheets or attachments if necessary.

Subterranean termites play a significant role in the ecosystem because they recycle woody materials. However, woody materials are, because of their chemical composition, difficult to degrade. Wood is composed of cellulose (40-50%) and lignins. Celluloses are high molecular weight, linear polysaccharides made up of D-glucopyranose units linked β-1→4. These polymers are difficult for many organisms to digest.

Two major groups of organisms possess the capability to degrade woody materials. These are free-living fungi, and the symbiotic microorganisms that live in the digestive tract of subterranean termites. Symbiotic microbes require the anaerobic environment provided in

termite hindgut to digest and convert wood materials into the byproducts useful to termite metabolism: smaller glycopyranose units which ultimately are degraded to hexoses (glucose).

Inulins and levans are fructans, which are either synthesized in plants or produced extracellularly by some bacteria. Inulins are polysaccharides comprised of D-fructofuranosyl units linked β-2→1. Inulins are found in the roots and tubers of plants in the family *Compositae* where they function as reserve polysaccharides. Inulins are relatively low molecular weight molecules, ranging from 20 to 30 D-fructofuranosyl units (3000 to 5000 Da) Levans are polysaccharides comprised of fructofuranosyl units linked β-2→6, and are found primarily in grasses. The levans have higher molecular weights than inulins (100 to 200 fructofuranosyl units). Inulins and levans are not easily degraded by many organisms. In fact, a primary use for inulin in human food is as a fiber additive (also a sugar substitute, fat replacer, or texturing agent) which has been classified as non-digestible oligosaccharides. The inability of these fructans to be degraded by many organisms indicates that they may have increased longevity in the soil (in-ground termite bait station) when compared to other consumption enhancing (sugar-like) compounds. However, the rate of degradation of these compounds in the natural environment is unknown.

Our disclosure is based on the hypothesis that plant-derived β-linked hexose polymers, such as the fructans, are feeding attractants for termites. This is because they represent less complex molecules than those found in natural woody materials and might be digested more easily by the termite microbial system. Support for this hypothesis is based on our discovery in choice test experiments that cellulose (paper) diets treated with very low concentrations of inulin are significantly more attractive to termite workers compared to other diet sources tested. Thus, we anticipate that (several/all) relatively lower molecular weight β-linked D-fructofuranosyl fructans: the inulins (3,000-5000 Da) and related larger (up to 12,000 Da) fructofuranosides composed of D-fructofuranosyl units linked β-2→1, and levans (16,600-33,200 Da), fructofuranosides composed of D-fructofuranosyl units linked β-2→6, have the potential to be subterranean termite feeding stimulants in termite baiting systems.

Currently, there are no attractants or consumption enhancement compounds available for use in subterranean termite baiting systems. Therefore, it takes weeks and often months for termites to begin feeding at the bait stations in significant numbers. The addition of these β-fructofuranoside polymers to the termites bait matrix would be a significant enhancement to these termite bait systems. The addition of compounds like inulin would cause termites to feed preferentially on the bait matrix rather than other local food resources, and influence those termite workers to recruit additional termites to the bait stations.

5. What is the existing technology/art to which you are comparing?

We are comparing our enhanced bait matrix to that of the Sentricon Termite Elimination System, Dow AgroSciences, the FirstLine Termite Baiting System, FMC Corporation, and the Exterra Termite Baiting System; Ensystex Co. These 3 systems dominate the national termite baiting market.

6. How does your INVENTION differ from present technology, what problems does it solve, or what advantages does it possess? (This should be written so someone skilled in the art can understand it.)

Competing food resources (natural or structural wood) in the same location as a bait system decreases termite consumption of bait. Inulins, levans and similar β-fructofuranoside polymers would serve as a food additive/attractant which could be added to the current termite bait matrices. These consumption enhancing compounds would make the bait more palatable than competing food resources. So termites feed on these enhanced baits preferentially, consuming more of the bait than other food resources.

Table 4. Comparison of termite population consumption (mg) of each diet in the individual choice tests to determine if there was preferential feeding (n=5). The subterranean termite ate *30 times* as much of the inulin treated bait as the hexaflumuron treated bait. Hexaflumuron is active ingredient in the Sentricon Termite Elimination System ™ and is not a feeding determent.

		Consumption (mg)		
Day	Competing Diets	(Mean ± SEM)	*t*-statistic	*P*-value
5	Hexaflumuron	0.2 ± 0.1 a	-20.647	<0.0001
	Inulin	6.0 ± 1.3 b		

[1]Student's *t*-test for $\mu = 0.5$ (SAS Institute 1999). Values of $P \leq 0.05$ were used to indicate significance.

Effects of Competing Food Sources on Subterranean Termite, *Reticulitermes spp.*, (Isoptera: Rhinotermitidae), Consumption of Hexaflumuron Treated Baits in Laboratory Assays

Abstract: Subterranean termite consumption of ^{14}C-hexaflumuron treated baits was compared in laboratory no-choice and choice tests. In no-choice tests groups of 100 termite workers consumed an average of 3 mg of ^{14}C-hexaflumuron bait in 2d and 11.4 mg in 5d. Termite consumption of ^{14}C-hexaflumuron bait was reduced in the presence of a competing food resource but this reduction was not significant at 2d. However, at 5d consumption of the ^{14}C-hexaflumuron bait was significantly reduced to only 0.2 mg in the presence of an ^{3}H-inulin treated food resource. Consumption comparisons of competing food resources in the choice tests were made to determine termite preference for particular food resources. In choice tests comparing ^{14}C-hexaflumuron and ^{3}H-inulin treated bait, consumption of the inulin bait was significantly greater (6.0 mg) than the hexaflumuron (0.2mg) at 5d, indicating a preference for the inulin treated bait.

Quantification of radioactive isotopes from individual termites was used to determine how much a single termite consumed of ^{14}C-hexaflumuron diet in the presence of a competing food source. Individual termite consumption reflected the population consumption results. ^{14}C-hexaflumuron consumption was significantly reduced from 56.9μg in the no-choice test to only 19.3μg in the presence of the control diet at 5d. ^{14}C-hexaflumuron consumption by individual termites was also significantly decreased when the diet was offered in the presence of an ^{3}H-inulin food resource. The reduction of ^{14}C-hexaflumuron consumption in the presence of ^{3}H-inulin at 5d, dropped from 56.9μg in the no-choice test to only 2.3μg in the choice test. Consumption comparisons of ^{14}C-hexaflumuron and ^{3}H-inulin in the choice tests indicated a significant preference for the inulin treated bait.

Key Words: ^{14}C-hexaflumuron, ^{3}H-inulin, *Reticulitermes spp.*, consumption

Over the last decade baiting systems have become a widely used method of subterranean termite control. In general, these bait systems work by individual stations being installed in the ground around the perimeter of a structure. The stations contain untreated wood monitors. Foraging termites in the area encounter the stations, enter, and consume the monitors. Ideally, these termites recruit additional workers to the station so that large numbers of termites begin to feed. The infested monitors are then replaced with a bait matrix containing a toxicant. The termites that have been recruited to the monitor now begin to feed on the active bait matrix. Subsequently, the baited workers pass the active ingredient on to additional termites in their colony via trophallaxis. In this way, large numbers of termites, and possibly the whole colony is affected by the toxicant and killed.

One of the most widely used termite baiting systems is the Sentricon® Termite Elimination System which was developed by Dow AgroSciences in 1994. This product has been marketed as a colony elimination system, meaning that once termites start eating the bait, they will carry enough of the active ingredient back to the colony to destroy the entire nest. The active ingredient in the Sentricon® system is hexaflumuron. Hexaflumuron [N-(((3,5-dichloro-4-(1,1,2,2-tetrafluroethoxy)phenyl)- amino)carbonyl)-2,6-diflurobenzamide] is a slow acting chitin synthesis inhibitor (Su et al. 1987; Nakagawa et al. 1992). The mean half life of hexaflumuron within a body of a termite is ~9 days thus foraging termites survive long enough after ingestion to transfer the toxicant to other members of the colony (Sheets et al. 2000). However, the speed at which a termite colony is eliminated is still dependent on the number of worker termites recruited to the bait station and how much of the bait they consumed.

There are a number of factors that influence the efficacy of bait systems. Some of these factors are related to termite behavior and others are related to the environment in which the bait systems are placed. For example, when foraging for food, termite feeding behaviors and food preferences vary. In general, subterranean termites consume dead wood and decaying wood litter (Wood 1978). However, the species of wood and its condition of decay has a significant affect on termite consumption (Smythe and Carter 1970). The wood characteristics, such as density, diameter, particle size and nutritional value will influence how much and how quickly termites will consume that particular piece of wood (Behr et al. 1972; La Fage and Nutting 1978; Wood 1978). Subterranean termites are also known to consume paper, cardboard, and other forms of processed cellulose. In many cases these processed cellulose sources are consumed preferentially over natural food resources because they are easier to ingest (Suiter et al. 2002). There is also natural variation in termite foraging pressure so that different termite colonies attack food and feed at different rates (Su and La Fage 1984; Lenz 1985). Different rates of feeding have been correlated with termite colony size, the caste proportions and the time of year (Esenther and Beal 1978; Becker 1962; Su and LaFage 1987; Forschler 1998). Seasonal variation in termite foraging and feeding behavior is related to several environmental conditions. Temperature (Smythe and Williams 1972; Haverty and Nutting 1974), moisture (Collins 1969), and ground cover (or the lack of ground cover) have well documented effects on termite foraging during the different seasons (Su and La Fage 1984; Lenz 1985; Cornelius and Osbrink 2001).

Arguably, the most significant influence on termite bait system performance is the presence of competing food sources. Bait stations have a relatively small presence (size)

in the outdoor environment. Subterranean termites are known to prefer larger food sources over smaller ones (Waller 1988; Lenz 1994; Cornelius and Osbrink 2001) and do not abandon a large food source to consume one of smaller size. Also, when bait stations are installed in the ground, subterranean termites are usually already feeding on a structure or some other food source in the area. Because termites are known to be faithful to an established food source and will not leave it until it is near depletion, there is often a considerable wait after installation before subterranean termites begin to attack bait stations (Heidecker and Leuthold 1984; Oi et al. 1996). Therefore, bait system efficacy is often hindered by the many food source options available in a natural environment.

Yet, it is well documented that bait systems do get attacked by subterranean termites in the field, even when large numbers of competing food resources may be present (Su 1993; DeMark et al. 1995; Forchler and Ryder 1996). What is not known is how much the presence of these competing food resources can reduce the consumption of termite bait. If an individual termite can consume only a finite amount of food in a given time, it stands to reason that a termite given a choice of two food resources would eat significantly less of each resource than it would of one resource with no competition. Alternatively, the same termite given a choice might consume only one resource if it was more palatable than the other. Although it would be extremely difficult to evaluate the effects of food source competition in the field, relative estimates can be made in a laboratory setting. The purpose of this research was to quantify the effects of competing food sources on subterranean termite consumption of hexaflumuron treated "bait". In this study, ^{14}C-hexaflumuron, ^{3}H-inulin, and control treated food resources were presented

alone or in combinations to evaluate bait consumption by groups of subterranean termites and individual termite workers.

Methods and Materials

Subterranean Termite Collection. Five wild populations of *Reticulitermes spp.* were collected from fallen wood in forested areas of Fairfax County (N38° 43.29' and W77° 30.93'), Montgomery County (N37° 12.46' and W80° 24.47'), Rockbridge County (N37°48.00' and W79° 25.00'), and Roanoke County (N37° 19.53' and W79° 58.53'), Virginia. Termites were harvested by placing the infested wood into large storage bins (70L; Sterlite®; Sterlite Corporation, Townsend, MA) on top of damp, recycled paper towels (Acclaim®; Fort James Corporation, Deerfield, IL). As the wood dried out the termites moved into the moist paper towels. The infested paper towels were then transferred to plastic storage containers (11.3L; Rubbermaid, Wooster, Ohio) containing vermiculite (500g; moistened 150% by weight (Lenz et al. 1987; Cherokee Vermiculite Horticultural Fine Cherokee Products, Jefferson City, TN). Subterranean termite containers were stored in complete darkness (~21°C and 97% RH) until needed for testing. Subterranean termites were tested within one month after being collected from the field.

Termite Diet Preparation. Recycled brown paper toweling (Acclaim®; Fort James Corporation, Deerfield, IL) was cut into squares (35 x 35 mm) and used as a diet substrate. The diet substrates were placed into glass Pyrex Petri dishes (100x 20mm; Corning Glass Works, Corning, NY) and dried overnight in a single wall, gravity convection, laboratory oven (60°C; Blue M SW-17TA; Blue M Electric Company; Blue Island, IL). After 24h, each diet substrate was taken out of the oven, numbered in the corner with a pencil and weighed on a balance (Mettler AE163; Lab Tech, Inc.) to the

nearest 0.1mg. After weighing, certain diets were treated with radiochemicals. Note: that hexaflumuron cannot be stored at temperatures greater than 50°C. Therefore, it was essential that the radioisotopes were only applied after the diets were oven dried. The weight of the isotopes on the diet after air drying was determined to be negligible.

Radiolabeled Chemicals and Visual Markers. Radioisotopes, C^{14}-hexaflumuron and H^3- inulin, technical grade hexaflumuron (non-radiolabeled), and Nile Blue-A (visual marker) were formulated for application to termite diet substrates. Radiolabels were used to quantify the effects of competing food sources on hexaflumuron consumption. Hexaflumuron-dichlorophenyl-UL-[^{14}C] (lot F0662-54, specific activity of 21.5mCi/mmol) was obtained from the radiosynthesis group at Dow AgroSciences (Indianapolis, IN). Radiochemical purity of the hexaflumuron had been determined by the manufacturer to be 98.7%. Nonradiolabeled, technical-grade hexaflumuron (98% pure) was also obtained from chemical resource services at Dow AgroSciences (lot# 17/95; Indianapolis, IN). Inulin-methoxy, [methoxy-^{3}H-] (lot 2978-124, specific activity 200mCi/g) was purchased from the radiosynthesis group at the DuPont Company (Wilmington, DE). Radiochemical purity of the inulin had been determined by the manufacturer to be 98.7% using high pressure liquid chromatography. Inulin is a widespread ß-linked carbohydrate (fructans) and is present in more than 30,000 plant species (Inulin Plaza 2003). In this study, inulin was used as an alternate control diet treatment to compete with hexaflumuron. Nile Blue-A (Allied Chemical Company, Morristown, NJ) was selected as the visual marker because of its long-term visibility and low associated mortality for *Reticulitermes flavipes* (Haagsma and Rust 1993; Oi and Su 1994; Su et al. 1991; King 2000; Suarez and Thorne 2000b). The purpose

of the dye was to ensure that termites were feeding on the diets. Termites that fed on the experimental diets were dyed a bright blue color.

Diet Treatments

Control Diets. Control diets were treated with the visual marker only. The visual marker was formulated by dissolving Nile Blue-A in acetone and applying a 150µl aliquot of dye solution to each diet substrate. The final Nile Blue-A concentration on the diet was 0.1%

^{14}C-hexaflumuron Diets. A stock solution of technical grade hexaflumuron, ^{14}C-hexaflumuron (0.5% total hexaflumuron concentration), and 0.1% Nile Blue-A was formulated in acetone. An aliquot (150µl) of the solution was applied to each diet substrate so that each diet contained 0.27 µCi of the ^{14}C-hexaflumuron.

^{3}H-inulin Diets. A stock solution of ^{3}H-inulin and 0.1% Nile Blue-A (1.95mg) was formulated in acetone. An aliquot (150µl) of the solution was applied to each diet substrate so that each diet contained 2.05 µCi of the ^{3}H-inulin. ^{3}H has a lower energy spectrum than ^{14}C, so ~8 x more ^{3}H-inulin was added to the diets than ^{14}C-hexaflumuron to facilitate radioisotope quantification process after termite consumption.

Sand Preparation: Approximately 4L of play sand (Quikcrete®; Quikcrete Companies, Atlanta, GA) was washed with tap water 4 times to remove impurities. Washed sand was dried for 48h in a single wall gravity convection laboratory oven (270°C; Thelco®; Precision PS Scientific, Chicago, IL) prior to use. The dried sand was then placed in a plastic storage bag (3.79L; Target Corporation, Minneapolis, MN) and moistened with distilled water (15% by weight of the sand). Moistened sand was then kneaded by hand in the storage bag and stored for at least 24h to ensure even distribution of moisture. Sand was stored in the plastic bag until needed for testing.

Bioassay Arenas: Consumption bioassays were performed in choice and no-choice bioassay arenas. Arena tests were used to evaluate termite consumption of hexaflumuron treated bait alone and in the presence of a competing food source.

No-Choice Bioassay. No-choice bioassay arenas were assembled by connecting two Petri dishes with Tygon tubing (inner diameter 3.2mm, outer diameter 6.4mm). Petri dishes were washed 4 times in tap water to eliminate static electricity. Tygon tubing was soaked in tap water for 3h and allowed to air dry prior to use. One Petri dish (95 mm x 15 mm; Fisher Scientific) served as a termite housing chamber and was filled with moist sand (~45g). A second, smaller Petri dish (60 mm x 15 mm; Fisher Scientific) served as a diet chamber. Termites placed in the no-choice arenas could forage from the housing chamber through the tubing to the diet chamber. .

Choice Bioassay. Choice arenas were similar to the no-choice arenas with the exception that the housing chambers were attached to smaller two diet chambers (60 mm x 15mm), each containing a different diet. Thus, termites placed inside choice arenas could simultaneously forage on two diets at once. Choice bioassays were set up in each of the following diet combinations: ^{14}C-hexaflumuron and control, ^{3}H-inulin and control, and ^{14}C-hexaflumuron and ^{3}H-inulin.

Bioassay Design.

Prior to testing, 100 worker termites (at least 3rd instar) were aspirated out of the storage containers and transferred into the housing chamber of one experimental arena. Termites were allowed to acclimate to the arena and forage on untreated paper towel for 72h. After the acclimation period, paper towel was removed, and all clinging termites

were gently tapped back into diet chamber. Experimental diets were then placed into the diet chambers.

For uniformity, all diets were secured in the Petri dishes. However, securing the diets was intended to minimize radioactive contamination of the arenas in tests using radiochemicals. Diets were secured by placing them on top of a glass coverslip (Rect. No.1 22 x 30cm; Corning Labware and Equipment) inside the diet chamber and putting 1/3 of a standard paper clip on top of them. A magnet (19.05mm Diameter; ProMag®, Marietta, OH) was placed underneath the bottom of the Petri dish holding the diet between the paper clip and the magnet. Three pieces of masking tape were put on the outside of the arena to seal the housing chamber lid.

Bioassay arenas were set up for each of the five treatment groups: 2 no-choice tests, either control or hexaflumuron, and 3 choice tests, hexaflumuron and a control, inulin and a control, or hexaflumuron and inulin. Each treatment was further subdivided into two test periods: 2d and 5d. Each test day within a treatment was replicated five times for a total of 50 arenas, 20 no-choice, and 30 choice. Each replicate within a treatment/day contained termites from a different field population. Once the data from an arena had been recorded on a particular test day, the arena was removed from the test.

Humidity Chamber. All bioassay arenas were placed inside humidity chambers, which were constructed by pouring play sand into the bottom of a plastic storage container (11.3L; Rubbermaid, Wooster, OH) to a depth of 4.5 cm. Tap water was poured over the sand to the point of saturation. Any standing water was absorbed with paper towels. A sheet of aluminum foil (Super Foil™; Atlantic Paper & Foil Corporation, Hauppauge, NY) was laid down to cover the saturated sand. Arenas containing termites were placed inside plastic

storage bags (3.8L; Target Corporation, Minneapolis, MN) and set on top of the aluminum foil in the chamber. The top of the plastic bag was rolled down to allow for air circulation. Plastic bags were used to separate the arenas from each other and to catch escaped termites. Escaped termites were returned to their respective experimental arenas. Humidity chambers were closed with snap top lids and were placed in total darkness in a cabinet (~21°C and 97% RH) for the duration of the experiment.

Recording Mortality. Termite mortality was recorded to ensure that the test insects were vigorous (Sheets et al. 2000), and that excessive mortality did not influence consumption data. Termites that did not seem to exhibit proper molting were considered moribund and were added to the mortality count (Su and Scheffrahn 1993).

Diet Consumption by Groups of Termites. After termite mortality was recorded for each bioassay, all partially consumed diets (no choice and choice) were removed from the arenas and oven dried for 24h. Diets were then weighed again to calculate consumption. Post-test weights of partially consumed diets were subtracted from the initial diet weights to determine total consumption by treatment.

Diet Consumption by Individual Termites. After mortality was recorded for a particular treatment/day, 20 surviving termites were randomly selected from each radioactive treatment arena for isotope analysis. Random selection was not limited to dyed termites. Non-dyed termites were also selected. The termites were thoroughly rinsed with distilled water three times to remove any radioactivity from the outside of the body. They were next placed on paper towels to remove excess water. Each rinsed, dried termite was placed into a separate microcentrifuge tube (1.5ml; Fisher Brand), and the body was homogenized in 200µl of distilled water. The homogenate was transferred

into a glass scintillation vial (20ml; Kimble Glass, Vineland, NJ). Microcentrifuge tubes were rinsed three times with 200µl of distilled water per rinse. Each rinse was added to the homogenate in the scintillation vial. An aliquot of scintillation cocktail (8 ml; Scintiverse® BD; Fisher Scientific, Fair Lawn, NJ) was then added to each vial.

The amount of radioactivity contained within each sample was quantified by using a scintillation counter (Beckman Coulter, Inc. ™ LS 6500, Fullerton, CA). The individual termite samples contained very low dpm counts. Therefore, sample vials containing only a single isotope (from no-choice tests) were counted for 10 minutes. However, sample vials that potentially could contain dual isotopes (from choice-tests) were counted for 20 minutes to improve the accuracy of the counts.

Background Radioactivity Measurements. After termite mortality had been recorded, 2 termites were selected at random from each no-choice, control arena. These termites were rinsed, homogenized, and prepared for scintillation counting as previously described. Control termites were counted in the scintillation counter for 10 minutes to record background radioactivity. Background radioactivity was measured for each treatment group (choice and no-choice) at both 2d and 5d. Background measurements also accounted for any quenching that occurred due to the termite body fragments occluding light transmission in the scintillation counter. Before the test data was analyzed, background radioactivity counts for a particular sample was subtracted from the sample's spectral peak (Traniello et al. 1985; Suarez and Thorne 2000a).

Radiolabel Counting using Dual Label Technique. Specialized procedures were required for analysis of radiolabeled samples containing both ^{14}C and ^{3}H. Due to the potential low/high counts and spillover of ^{14}C and ^{3}H in the counting window, a series

of dilutions was made from extracted diets (with known amounts of activity) to correct for spillover. The range of activity values for ^{14}C and ^{3}H and a combination of the two isotopes within the samples was determined. Spillover correction curves were generated for the range of sample data and used to correct those samples where high levels of both ^{14}C and ^{3}H occurred and spillover was evident. This procedure was developed after consultation with Beckman Coulter, Inc.™ technical support group.

Statistical Analysis. Bioassays were arranged in a 5 (treatment) by 2 (day) factorial, randomized complete block design (RCBD, SAS Institute 1999). The data was blocked by termite field population to account for within treatment variability due to differential feeding between colonies.

The mean percentage of termite mortality (to assure termite vigor) between the 5 bioassay treatments was analyzed separately for tests run for 2d and 5d. Mortality was compared using analysis of variance (ANOVA, SAS Institute 1999). Values of $P \leq 0.05$ were used to indicate significance.

Comparisons of termite diet consumption (mg) between treatments were analyzed separately at 2d and 5d using ANOVA. The model for the ANOVA used termite colony and treatment as sources of variation.

Mean consumption of the hexaflumuron diet in the no-choice tests was compared with the mean amount of hexaflumuron diet consumed in the presence of a competing food source (choice tests: hexaflumuron and control or hexaflumuron and inulin) using the Student's *t*-test for both 2 and 5 days respectively (SAS Institute 1999).

Mean consumption of the inulin diet in choice tests, inulin and control or inulin and hexaflumuron, was compared using ANOVA. The model for the ANOVA used the colony and treatment as sources of variation.

Finally, mean consumption of the two diets within each of the three choice tests was analyzed to determine whether termites preferred one diet over another. Due to the binomial nature of the choice test design, diet consumption was analyzed using a Student's *t*-test for $\mu = 0.50$ (SAS Institute 1999). For all consumption tests values of $P \leq 0.05$ were used to indicate significance.

Quantification of radioactive isotopes from individual termite cadavers was used to determine how much a single termite consumed of ^{14}C-hexaflumuron diet in the presence of a competing food source. Mean consumption of ^{14}C-hexaflumuron diet in the no-choice test was compared with ^{14}C-hexaflumuron consumption in the choice test using GLM ANOVA with Least Squares adjustment for multiple comparisons of mean values. For all tests data was analyzed separately for 2d and 5d.

Although the ^{3}H-inulin had been provided simply as a food source alternative to the hexaflumuron treated bait, termite consumption of the ^{3}H-inulin diet was surprisingly high by comparison. Therefore, as a point of interest, the mean consumption of ^{3}H-inulin diet in the two choice tests, ^{14}C-hexaflumuron and ^{3}H-inulin, and ^{3}H-inulin and control, were compared using GLM ANOVA (SAS Institute 1999).

Finally, mean consumption of the two radioisotopes, ^{14}C-hexaflumuron and ^{3}H-inulin, in the choice tests were compared to determine whether termites preferred one diet over another. Mean consumption was analyzed using a Student's *t*-test for $\mu = 0.50$ (SAS Institute 1999).

Because termites that did not contain the visual marker were used in the radioisotope consumption analysis as well as those that did, variability in the consumption data was relatively high. We would expect this variability among termites in the field to be even more pronounced. Therefore, we used values of $P \leq 0.1$ to indicate significance for all consumption studies evaluating individual termites.

Results

Mortality. Subterranean termite mortality was low across all treatments with an average of less than 3% at 5d (Table 1). Mortality was not significantly different between any of the treatments at 2d ($P = 0.598$) or 5d ($P = 0.974$).

Diet Consumption by Termite Populations. Mean consumption (mg) by the subterranean termite populations was not significantly different for any of the treatment groups, either choice or no-choice at 2d ($P = 0.714$; Table 2). Overall, at 2d termites ate the same amount (~3mg) in the individual no-choice arenas as they did in the choice tests where consumption of the two diets was quantified together. As expected, the termites had eaten considerably more diet by 5d, however, consumption of diets between the treatment groups was still not significantly different (~ 11 mg; $P = 0.148$).

To determine the impact of competing food sources on hexaflumuron consumption, the quantity of the hexaflumuron diet consumed in the no-choice tests was compared with the quantity of diet consumed in the choice tests, where the hexaflumuron was competing with either a control diet or and inulin treated diet (Table 3). Mean consumption of hexaflumuron in the no-choice tests was 3.0 mg at 2d (Table 2). In the choice tests where the hexaflumuron diet was offered with the control diet, consumption of the hexaflumuron diet was reduced to 1.6mg at 2d. This reduction was not significant

($P = 0.170$). When the inulin diet was offered as a competing food source, the hexaflumuron diet consumption was reduced to 1.0mg at 2d. This reduction was also not significant ($P = 0.068$). At 5d consumption of hexaflumuron in the no-choice test was 11.4mg. In choice tests where the hexaflumuron diet was competing with the control diet termite consumption of hexaflumuron was reduced to 3.2mg ($P = 0.080$). This reduction was not significant. However, in the choice test where the inulin diet was competing with the hexaflumuron diet, consumption of hexaflumuron was significantly reduced to only 0.2mg at 5d ($P = 0.045$).

Finally, paired comparisons of diet consumption within the three choice tests were made to determine if the termite populations preferred one diet over another. Preference was defined as the consumption of one diet in the choice test being significantly greater than 50% of total consumption. At 2d, termite diet consumption indicated no feeding preferences for either diet offered in any of the choice tests (Table 4). However, at 5d a preference for the inulin diet was indicated in one of the choice tests. Termites consumed significantly more of the inulin diet (6.0 mg; $P < 0.0001$) than the hexaflumuron diet (0.2 mg) at 5d. However, it is interesting to note that the inulin diet was not preferred over the control diet ($P = 0.333$) nor was the control diet preferred over the hexaflumuron diet in the choice tests ($P = 0.841$).

Diet Consumption by Individual Termites. When the average ^{14}C-hexaflumuron consumption by and individual termite was compared in no-choice and choice tests the differences were significant ($F = 7.76$, $df = 1$, $P = 0.017$). At 2d ^{14}C-hexaflumuron consumption was significantly reduced from 38.9µg in the no-choice test to only 14.5µg in the choice tests where ^{14}C-hexaflumuron was competing with the

control diet ($P = 0.090$; Table 5). Similarly at 5d, ^{14}C-hexaflumuron consumption was significantly reduced from 56.9μg in the no-choice test to only 19.3μg in the presence of the control diet ($P = 0.058$).

Like the control choice tests, consumption of ^{14}C-hexaflumuron by individual termites was significantly decreased when the diet was offered in the presence of an ^{3}H-inulin food resource ($F = 48.89$, $df = 1$, $P < 0.0001$). At 2d ^{14}C-hexaflumuron consumption was significantly reduced from 38.9μg in the no-choice test to only 7.3μg when competing with the ^{3}H-inulin diet ($P = 0.001$). The reduction of ^{14}C-hexaflumuron consumption in the presence of ^{3}H-inulin was even more pronounced at 5d, where consumption was dropped from 56.9μg in the no-choice test to only 2.3μg in the choice test ($P < 0.0001$).

Finally, the consumption of the ^{14}C-hexaflumuron and ^{3}H-inulin were compared in the choice test to determine if single termites preferred one diet over the other. At 2d the consumption data indicated that the termites consumed some of both diets but did not prefer either diet ($P = 0.285$; Table 3-6). However, by 5d termite consumption indicated a significant preference for ^{3}H-inulin ($P = 0.002$). On average, individual termites consumed 19.4μg of ^{3}H-inulin diet and only 2.3μg of ^{14}C-hexaflumuron diet.

Discussion

Several of the factors in our experimental design had the potential to negatively impact the health of the termites and subsequently influence consumption. Because the rate of food consumption by healthy subterranean termite colonies is known to be significantly greater than the consumption rate of termites with a high percentage of mortality (Su and La Fage 1984), it was important to determine that the termite

populations used in these laboratory assays were not negatively affected by disease or handling. It was also important to ensure that the termites were not yet intoxicated by the hexaflumuron or poisoned by the radioisotopes and thus not consuming food at a normal rate.

Termite mortality in this study was less than 3% for all treatment groups. Therefore, handling and disease did not negatively impact the termite populations or reduce consumption in any of the treatment groups. In addition, previous studies determined that the LT_{50} for subterranean termites fed hexaflumuron bait was ~26.6d and that the onset of toxicity does not occur until approximately 15d (Sheets et al. 2000). The 5 day duration of the consumption experiments described here fall well short of the expected onset of hexaflumuron toxicity. Termite mortality was not significantly different between any of the treatment groups, those that contained radioisotopes and those that did not. Therefore, we were able to conclude that differences in consumption between the treatment groups were not influenced by mortality or morbidity due to any of the dangers inherent in the research design.

Although termite vigor is one of the most important influences on consumption in the laboratory environment, influences on termite feeding behavior are far more complex in the field, and warrant some discussion here. There are several major factors that have been documented to influence subterranean termite food choice and consumption. For example, previous feeding history of the colony can affect new food choices (McMahan 1966; Heidecker and Leuthold 1984; Wood 1978). A termite colony may become preconditioned to prefer a particular food source rather than choosing to consume a new food source which is preferred by termites of the same species. Termites are also known

to prefer food that has been previously damaged by conspecific consumption and has a high (relatively) moisture content (Delaplane and La Fage 1989). Feeding behavior can also vary with colony size. For instance, foraging distance is limited in smaller colonies, because fewer workers are available to look for food. Therefore, smaller colonies may feed on only 1 or 2 available, but not preferred, food sources (Wood 1978). All of these factors need to be considered when interpreting laboratory assays for potential application in the field.

In this laboratory experiment, termites were exposed to only 1 or 2 food sources at a time, thus limiting the number of the potential influences on diet consumption. However, we were able to make some useful observations about termite consumption of the treated diets. First, we were able to concur with Su and Scheffrahn (1993, 1996) and King and Karr (2000) that the hexaflumuron diet, at the 0.5% concentration (w/w; Sentricon® % A.I.), caused no feeding deterrence and was just as palatable to the termites as the control diet. We also found that mean termite consumption across the five colonies was approximately the same for all treatments (hexaflumuron diet, control diet, inulin diet, or some combination) at 2d and at 5d.

However, because the termites ate approximately the same amount of diet in all the treatments, our results indicated that the termites could only eat a certain amount of diet in a given time. When two equally palatable food sources were offered, the termites ate less of each diet. Therefore, even though the hexaflumuron diet was palatable, its consumption was reduced in the presence of a competing food source.

In the choice tests where consumption of both diets offered were compared to see if there was a preference, the termites significantly preferred to consume the inulin over

the hexaflumuron diet. This result had not been anticipated. However, this preference did indicate that hexaflumuron consumption could be considerably influenced (reduced) by the particular type of food resource with which it was competing.

Radiolabels have been used successfully to study the metabolism of bait system toxicants within individuals and populations of termites. Radiolabels have been used to study trophallaxis (Alibert 1959; Gösswald 1963; Afzal 1983, 1984; Traniello et al. 1985; Rosengaus et al. 1986; Suarez and Thorne 2000b), foraging patterns (Easey 1981), and feeding behaviors in termites (Gösswald 1962; McMahan 1962, 1963, 1966). Sheets et al. (2000) used radiolabels to determine the rate of uptake, clearance, insect-to-insect transfer, and metabolism of ^{14}C-hexaflumuron in *R. flavipes*. In the consumption evaluation described in this study, the use of the radiolabeled diets allowed us to compare the consumption behavior of an individual termite worker to that of the test population.

The quantification of radiolabels within individual termites determined that individual consumption was a good indicator of how the population fed as a whole. Similar to the tests evaluating population consumption, the average termite sampled in a ^{14}C-hexaflumuron choice test contained less of the ^{14}C-radiolabel than a termite sampled from a no-choice test. Likewise, the individual termite consumption in the dual labeled choice test, ^{14}C-hexaflumuron and ^{3}H-inulin, further indicated that when given a choice, an individual termite consumed less of the ^{14}C-hexaflumuron than it did when there was no competing food resource. Also, consumption analysis in the dual label choice test indicated that the individual termites had fed on both the ^{14}C-hexaflumuron and ^{3}H-inulin diets. However, a comparison of the amount of each diet consumed indicated that, like the population as a whole, individual termites preferred to consume the ^{3}H-inulin diet.

Thus, we concluded that when the termite population was feeding from both diets in the choice tests, individual termites also fed from both diets, rather than a portion of the workers feeding from one diet, and another portion feeding from the other. Further, we observed that if the population consumed both diets but preferred to consume one diet over the other, individual termites exhibited the same diet preference.

In the field, subterranean termites are known to feed from multiple food resources (Grace and Su 2001), and a single termite population might be feeding on several food resources at once. However, the palatability of each available resource influences the amount of termite consumption on that resource. For example, subterranean termites have been documented to feed on what are known to be less preferred food items in their environment. However, their consumption of those food resources is diminished when a more desirable resource has been located (Smythe and Carter 1970).

Because a single termite has a limited consumption capacity and cannot consume more than this amount, the total amount of food a termite population can consume in a given period is also limited. As we found in the choice assays, consumption of more than one food source at a particular time resulted in all food resources being consumed less than when only a single food source was available. Further, the impact of food resource competition on a particular food was even more pronounced when the termites found the competitor to be more palatable.

These results suggest that as field populations of termites expand their foraging territories and discover new food resources, consumption of established food sources might be considerably reduced. Thus the influence of competing food resources on the efficacy of subterranean termite baiting systems in the field could be quite significant. If

a termite population established a bait station as a suitable food resource, the amount of bait consumed and its subsequent efficacy would not only be dependant on how many workers were recruited to the bait station, but also how many other food sources the colony was currently consuming and the palatability of any food resources the termites located in the future.

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Table 1. Mean percent mortality at 2d and 5d for subterranean termite populations fed different diets (no-choice tests) or diet combinations (choice tests).

Day	Treatment Group	n	Percent Mortality (Mean ± SEM)	*F*-statistic	*P*-value
2	Control Only	5	0.01 ± 0.01[1]	0.71	0.598[1]
	Hexaflumuron Only	5	0.01 ± 0.01		
	Hexaflumuron & Control	5	0.02 ± 0.01		
	Inulin & Control	5	0.02 ± 0.01		
	Hexaflumuron & Inulin	5	0.03 ± 0.02		
5	Control Only	5	0.02 ± 0.01	0.12	0.974
	Hexaflumuron Only	5	0.03 ± 0.02		
	Hexaflumuron & Control	5	0.02 ± 0.01		

Inulin & Control	5	0.03 ± 0.02
Hexaflumuron & Inulin	5	0.02 ± 0.01

[1]GLM ANOVA (SAS Institute 1999). Values of $P \leq 0.05$ were used to indicate significance.

Table 2. Mean total consumption (mg) of diets by subterranean termite populations fed different diets (no-choice tests) or diet combinations (choice tests).

Day	Treatment Group	n	Total Consumption (mg) (Mean ± SEM)	*F*-statistic	*P*-value
2	Control Only	5	3.2 ± 0.3[1]	0.53	0.714[1]
	Hexaflumuron Only	5	3.0 ± 0.7		
	Hexaflumuron & Control	5	3.9 ± 0.9		
	Inulin & Control	5	2.7 ± 0.6		
	Hexaflumuron & Inulin	5	3.1 ± 0.4		
5	Control Only	5	13.6 ± 2.1	1.97	0.148
	Hexaflumuron Only	5	11.4 ± 3.9		
	Hexaflumuron & Control	5	9.6 ± 3.1		

Inulin & Control	5	11.3 ± 3.2
Hexaflumuron & Inulin	5	6.2 ± 1.3

[1]GLM ANOVA (SAS Institute 1999). Values of $P \leq 0.05$ was used to indicate significance.

Table 3. Comparison of hexaflumuron consumption (mg) by termite populations in choice and no-choice tests at 2d and 5d.

Day	Test	Diet	n	Hexaflumuron Consumption (mg) (Mean ± SEM)	*t*-statistic	*P*-value
2	No-Choice	Hexaflumuron	5	3.0 ± 0.7a	2.80	0.170[1]
	Choice	Hexaflumuron	5	1.6 ± 0.9a		
	Choice[2]	*Control*	*5*	*2.2 ± 0.8*		
	No-Choice	Hexaflumuron	5	3.0 ± 0.7a	6.18	0.068
	Choice	Hexaflumuron	5	1.0 ± 0.8a		
	Choice	*Inulin*	*5*	*2.0 ± 0.6*		

5	No-Choice	Hexaflumuron	5	11.4 ± 3.9a	5.44	0.080
	Choice	Hexaflumuron	5	3.2 ± 1.4a		
	Choice	*Control*	*5*	*6.4 ± 3.8*		
	No-Choice	Hexaflumuron	5	11.4 ± 3.9a	8.32	0.045
	Choice	Hexaflumuron	5	0.2 ± 0.1b		
	Choice	*Inulin*	*5*	*6.0 ± 1.3*		

[1]Student's *t*-test (SAS Institute 1999). Means followed by different letters are significantly different ($P \leq 0.05$).

[2]Italics denotes consumption of competing diet. However, consumption of competing diet in choice test was not included in the statistical analysis.

Table 4. Comparison of termite population consumption (mg) of each diet in the individual choice tests to determine if there was preferential feeding.

Day	Choice Test	Competing Diets	n	Diet consumption (mg) (Mean ± SEM)	*t*-statistic	*P*-value
2	Hexaflumuron & Control	Hexaflumuron	5	1.6 ± 0.9 a[1]	-0.88	0.430[1]
		Control	5	2.2 ± 0.8 a		
	Inulin & Control	Inulin	5	1.3 ± 0.3 a	0.65	0.553
		Control	5	1.4 ± 0.7 a		
	Hexaflumuron & Inulin	Hexaflumuron	5	1.0 ± 0.8 a	-1.22	0.288
		Inulin	5	2.0 ± 0.6 a		
5	Hexaflumuron & Control	Hexaflumuron	5	3.2 ± 1.4 a	-0.22	0.841
		Control	5	6.4 ± 3.8 a		

Inulin & Control	Inulin	5	8.9 ± 3.7 a	1.099	0.333
	Control	5	2.3 ± 1.3 a		
Hexaflumuron & Inulin	Hexaflumuron	5	0.2 ± 0.1 a	-20.647	<0.0001
	Inulin	5	6.0 ± 1.3 b		

[1]Student's t-test for $\mu = 0.5$ (SAS Institute 1999). Values of $P \leq 0.05$ were used to indicate significance.

Table 5. Comparison of ^{14}C-hexaflumuron consumption (μg) by and individual termites in choice and no-choice tests.

Day	Test	Diet	n	Hexaflumuron Consumption (μg) (Mean ± SEM)	Hexaflumuron Consumption (μg) (LS MEAN)	*P*-value
2	No-Choice	^{14}C-Hexaflumuron	5	38.9 ± 2.9[2]	3.31 a[1]	0.090[1]
	Choice (Control)	^{14}C-Hexaflumuron	5	14.5 ± 1.7	2.16 b	
	No-Choice	^{14}C-Hexaflumuron	5	38.9 ± 2.9	3.31 a	0.001
	Choice (^{3}H-Inulin)	^{14}C-Hexaflumuron	5	7.3 ± 1.2	1.36 b	
5	No-Choice	^{14}C-Hexaflumuron	5	56.9 ± 4.3	3.77 a	0.058
	Choice (Control)	^{14}C-Hexaflumuron	5	19.3 ± 1.7	2.47 b	

No-Choice	^{14}C-Hexaflumuron	5	56.9 ± 4.3	3.77 a	<0.0001
Choice (^{3}H-Inulin)	^{14}C-Hexaflumuron	5	2.3 ± 0.1	1.17 b	

[1]Least Squares Method (SAS Institute 1999)

[2]Denotes the consumption data from which the Least Squares means were calculated. Mean followed by different letters are significantly different ($P \leq 0.05$).

Table 6. Comparison of diet consumption (μg) by individual termites in hexaflumuron and inulin choice tests to determine if there was preferential feeding.

Day	Test	Competing Diets	n	Diet Consumption (μg) (Mean ± SEM)	*t*-statistic	*P*-value
2	Choice	^{14}C-Hexaflumuron	5	7.3 ± 1.2a	-1.23	0.285[1]
		^{3}H-Inulin	5	12.6 ± 1.0a		
5	Choice	^{14}C-Hexaflumuron	5	2.3 ± 0.1a	-7.26	0.002
		^{3}H-Inulin	5	19.4 ± 1.2b		

[1]Student's *t*-test, for $\mu = 0.5$ (SAS Institute 1999). Means followed by different letter